Rubisco recovery from alfalfa juice by ion-exchange chromatography in expanded bed

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LABORATOIRE D'INGÉNIERIE
DES SYSTÈMES BIOLOGIQUES
ET DES PROCÉDÉS

PLAN

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III. Material and methods

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I. Introduction

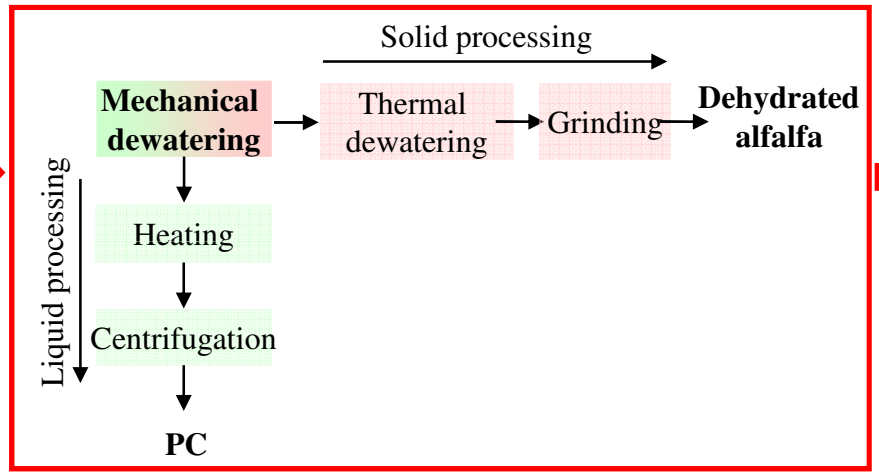
Biomass

Alfalfa

33 millions ha
(700 000 in France)

~ 2500 kg proteins/ha
(3x soybean, 4x wheat)

Fractionation process



Proteins concentrate (1%) **+**

Feed

Dehydrated alfalfa (99 %)

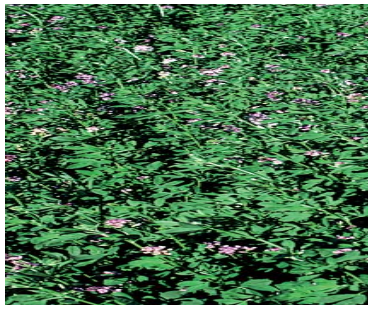
France : 800 000 t/year

⊖ **Low added value**

⊖ **Disadvantageous energy balance**

I. Introduction

Biomass



Alfalfa

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~ 2500 kg proteins/ha
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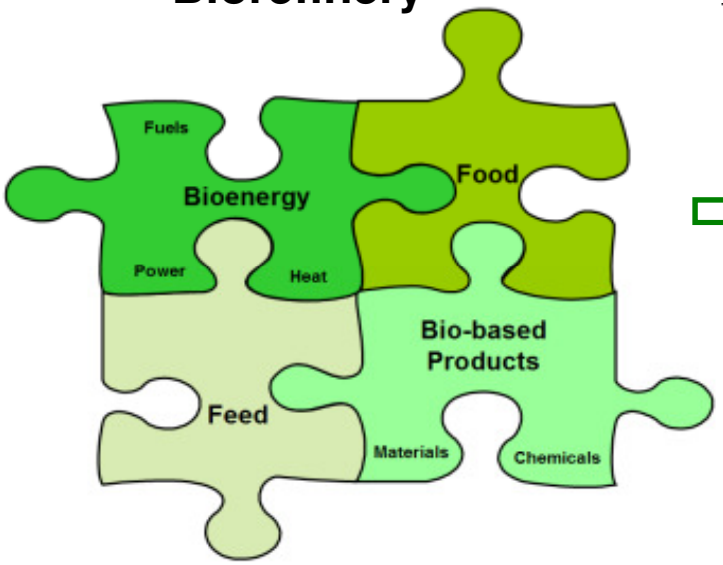
Biorefinery



⊕ **Diversification**

⊕ **Added value**

⊕ **Energy input**

⊕ **Pollution**

Products

Proteins, amino acids
ethanol, organic acids
Cellulose, enzymes,
biofuels …

Dehydrated alfalfa

II. Objectives

Leaf proteins are **abundant** and **renewable**

Proteins in alfalfa juice : up to **20%** of DM :

- 50% **hydrophobic** proteins
- 50% **hydrophilic** proteins

Rubisco : Ribulose 1,5 Biphosphate Carboxylase Oxydase

- 70% of **hydrophilic** proteins
- **Valuable** in many fields (human nutrition, pharmaceuticals, environmental …)

Aim

Study of Rubisco recovery from alfalfa juice by ion-exchange chromatography in expanded bed

Preliminary approach :

- Centrifuged alfalfa juice
- Different Rubisco content

II. Objectives

Crude extract

Primary purification

Liquid-solid separation:
- Centrifugation
- Filtration

Concentration

Primary separation:
- Chromatographic processes
- Adsorption
- Membrane filtration

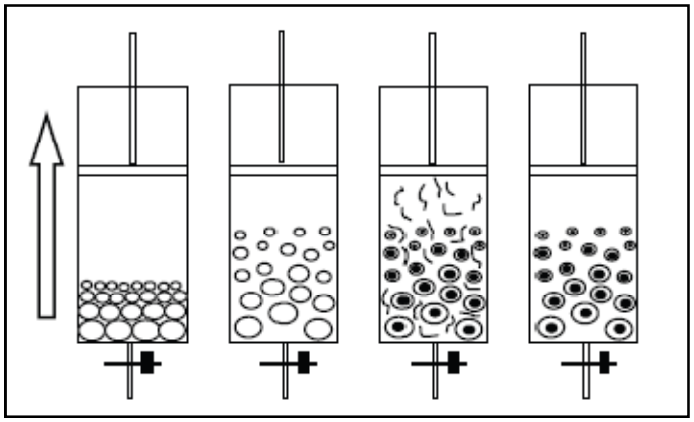
High resolution

Purification

Polishing

Pure product

Expanded Bed Chromatography (EBC)



Ion exchange
- Good selectivity
- High binding capacity

+

Expanded Bed
- High treatment capacity

Interesting alternative

III. Material and methods

Vegetable material

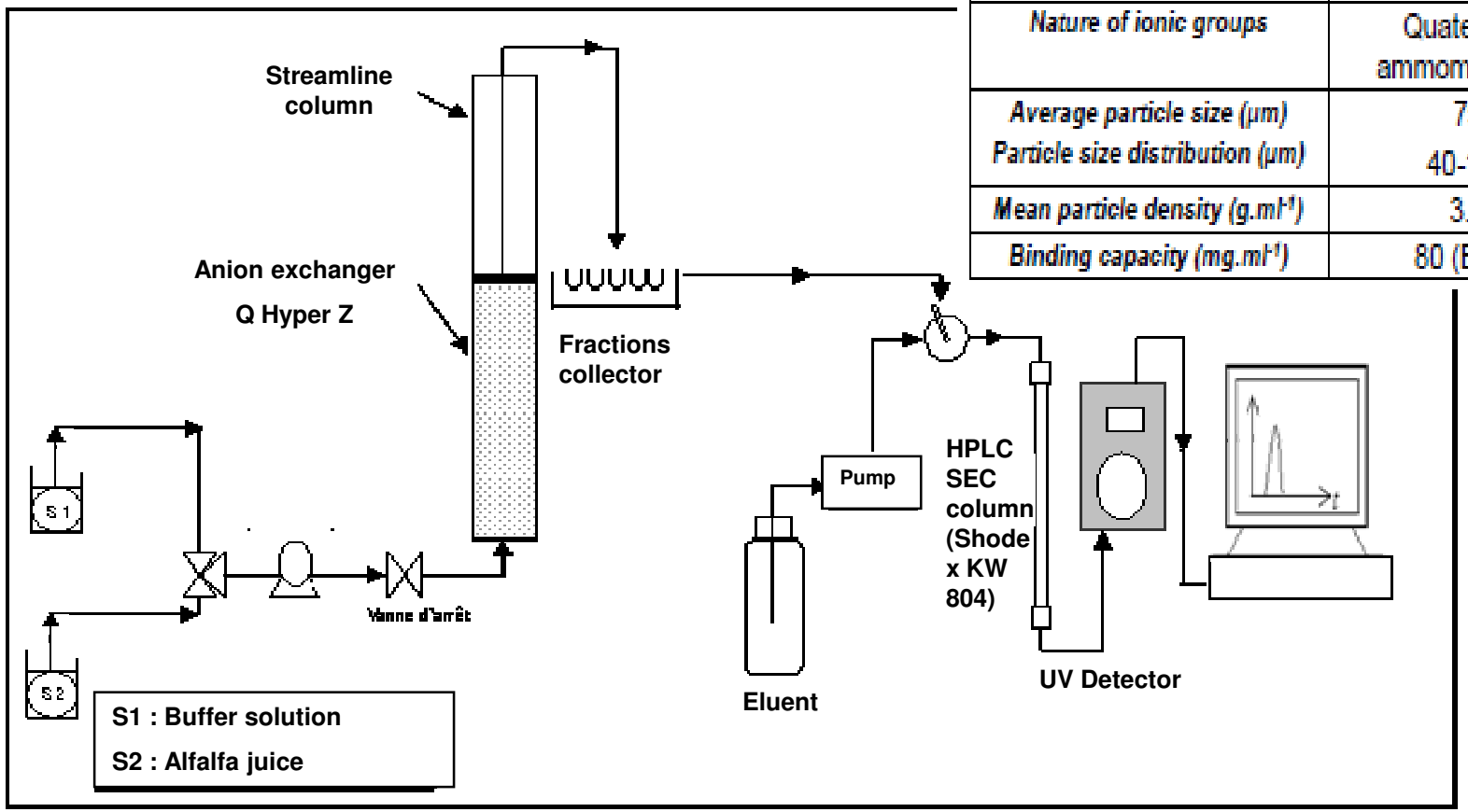
Physico-chemical properties of centrifuged alfalfa juice

	Centrifuged juice
pH	5.8
Conductivity ($mS.cm^{-1}$)	17.0
Viscosity (Pa.s)	2.10^{-3}
Density ($kg.m^{-3}$)	1042
Dry matter (%)	8.1
Total nitrogen content ($g.L^{-1}$)	19.43
Hydrophobic proteins content ($g.L^{-1}$)	1.84
Hydrophilic proteins content ($g.L^{-1}$)	16.92
Rubisco content ($g.L^{-1}$)	13.3

III. Material and methods

Experimental set-up

	Q Hyper Z
Nature of ionic groups	Quaternary ammonium (Q)
Average particle size (µm)	75
Particle size distribution (µm)	40-105
Mean particle density (g.ml⁻¹)	3.2
Binding capacity (mg.ml⁻¹)	80 (BSA)



III. Material and methods

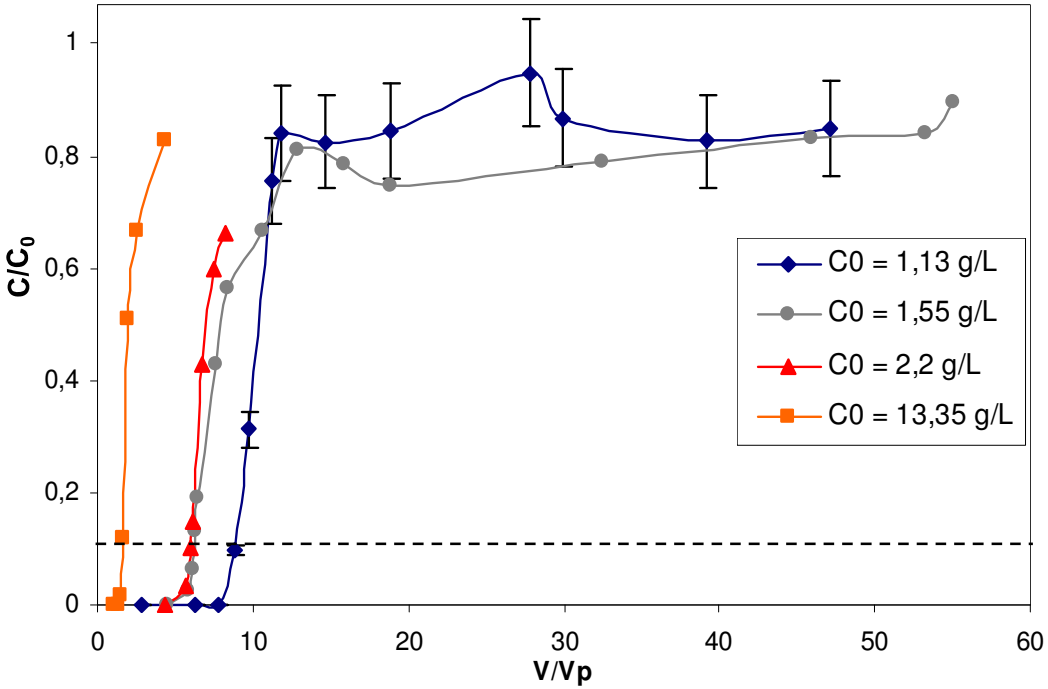
Operating conditions

	Charge			Elution	
	C_0 ($g.L^{-1}$)	Dilution	Superficial velocity ($cm.min^{-1}$)	Concentration of NaCl ($mol.L^{-1}$)	Superficial velocity ($cm.min^{-1}$)
Experiment 1	1.13	1:10	5.3	0.5	2.65
Experiment 2	1.55	1:8			0.65 (fixed bed mode)
Experiment 3	2.20	1:6			0.40 (fixed bed mode)
Experiment 4 (Centrifuged raw juice)	13.35	1:1			0.40 (fixed bed mode)

H = 21 cm ***$\Phi_{colonne}$= 2.5 cm***

VI. Results

Breakthrough curves



Experimental breakthrough curves for Rubisco obtained for a Q Hyper Z column in expanded bed mode.

	Conductivity ($mS.cm^{-1}$)	$Q_{10\%}$ ($g_{Rubisco}.L^{-1}{}_{QHyper\ Z}$)
Experiment 1	1.22	20.1
Experiment 2	1.43	19.2
Experiment 3	2.11	24.8
Experiment 4	17.00	17.8

- C_0 Rubisco ↗ $Q_{10\%}$ ↗ even if conductivity ↗

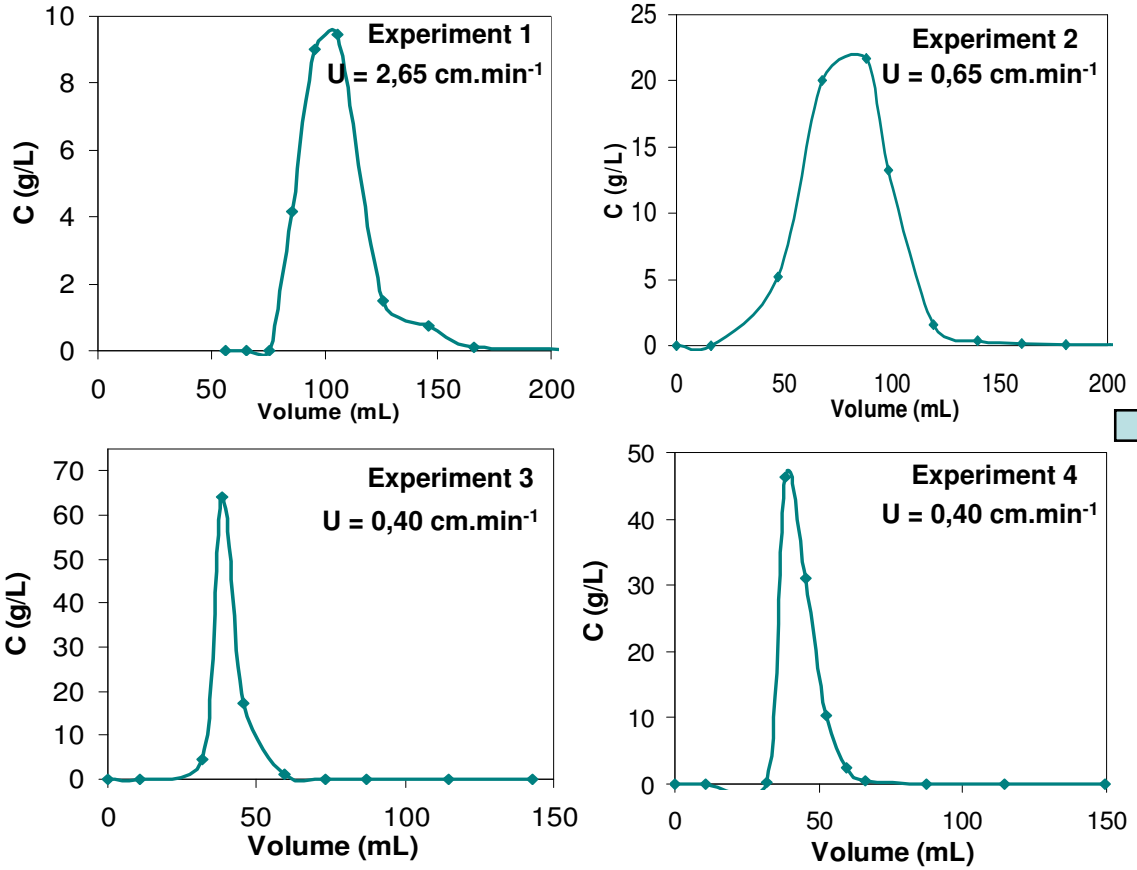
- $Q_{10\%}$ = 37 $g_{BSA}/L_{Q\ Hyper\ Z}$ *(Vergnault, 2004)*
  = 35.9 $mg_{BSA}/ml_{Q\ Hyper\ Z}$ *(Xia et al., 2007)*

Determination of dynamic capacity :

$$Q_{10\%} = \frac{C_0\left(V_{10\%} - V_p\right) - \int_0^{V_{10\%}} C \cdot dv}{V_R}$$

VI. Results

Elution curves

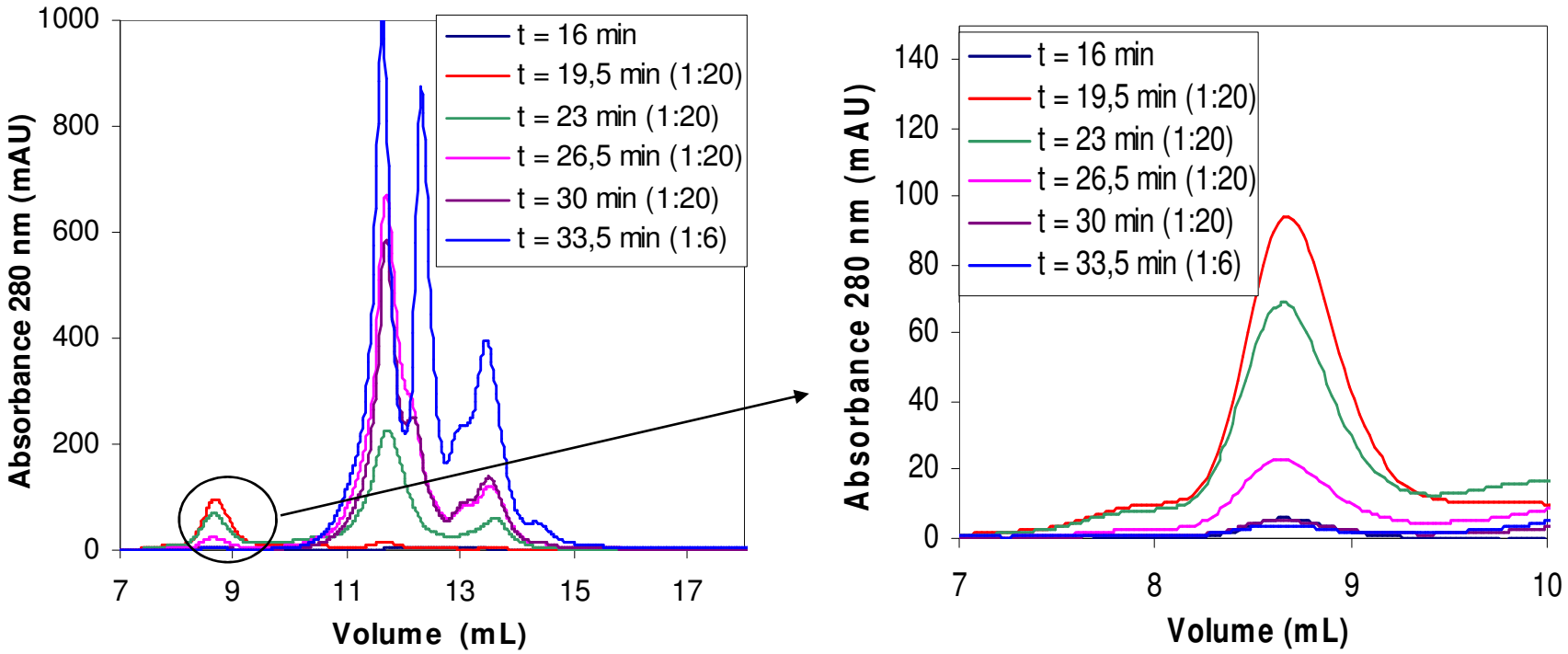


- Elution velocity ↘ , thin elution peak
- High concentration of eluted fraction : up to 60 g/L

Elution curves of Rubisco performed with 0.5 M NaCl at different superficial velocities

VI. Results

Qualitative analysis

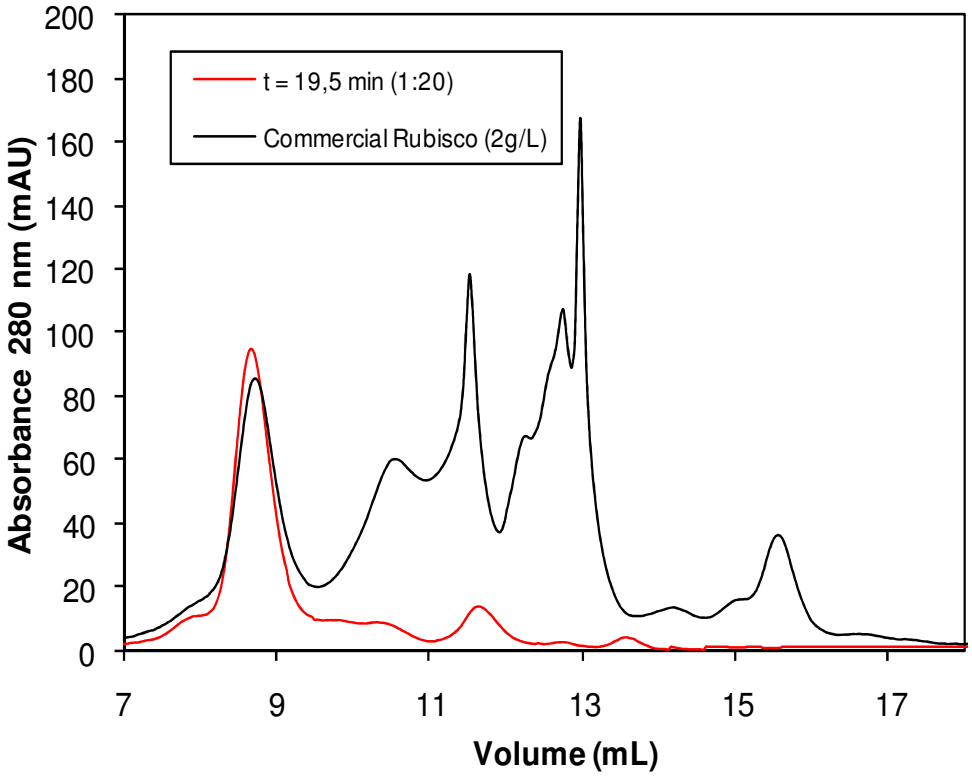


Chromatograms obtained by HPLC SEC analysis of eluted fractions (experiment 3).

- High concentration of Rubisco
- Absence « contaminants » peaks in the first eluted fraction

VI. Results

Qualitative analysis

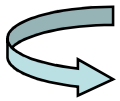


Production of Rubisco fraction at very high concentration and purity when compared with available Rubisco

Comparison between chromatograms obtained by HPLC SEC analysis of commercial purified Rubisco and fraction 5 obtained by EBC process

V. Conclusions and perspectives

- Rubisco can be separated by a simple process with good performances.
- Even the complexity and the high conductivity of the juice, $Q_{10\%}$ obtained are good.
- Dilution of the juice before EBC treatment could be interesting in order to reduce its ionic strength and increase the dynamic capacity of the adsorbent.
- Fixed Rubisco is easily eluted at 0.5 M NaCl.
- Elution in fixed bed mode at low velocity allows the recovery of high concentration Rubisco fractions with high purity.



- Experiments without centrifugation step
- Scale up
- Rubisco binding mechanisms

THANK YOU FOR YOUR ATTENTION

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